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Preparation and charecterization of PEG nanoparticle loaded with Irinotican hydrochloride

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ABSTRACT

Irinotecan Hydrochloride is an anticancer drug, a potent inhibitor of enzyme topoisomerase-1 activity. Its absorption is quite rapid in GIT, having a biological half life of about 6 – 12 hrs. The present aim of the work was to formulate nanoparticles of irinotecan hydrochloride using poly ethylene glycol as drug carrier and to evaluate the prepared formulation. Irinotecan loaded nanoparticles were prepared by the emulsification solvent evaporation method. The characteristics of formed nanoparticles were evaluated by using SEM, DSC and In-Vitro release kinetics. The formulation shows better percentage of drug release of about 81 % over a period of 24 hours.

Keywords: Potent inhibitor, Absorption, Emulsification, carrier, Controlled release.

Abbreviations: NP – Nanoparticle, DNA - Deoxy ribo nucleic acid, PEG - Poly ethylene glycol, DSC - Differential Scanning Colorimetry, SEM - Scanning Electron Microscopy, DM water - De Mineralised water, GIT - Gastro Intestinal Tract, UV - Ultra Violet spectroscopy, rpm - Revolutions per minute.

Antineoplastic:
An agent or drugs that prevent or inhibit the development of neoplasms, ie, inhibiting the growth of malignant cells.

Biodegradable polymer:
Biodegradable polymers are polymers that break down and lose their initial integrity then finally degrade within the biological fluids. These polymers are used in medical devices to avoid a second operation to remove them, and it also could be implemented in drug delivery. The drug slowly releases in a controlled manner as polymer degrades.

Nanospheres:
Nanospheres are the biodegradable, self assembling spherical particle whose diameter is measured in nanometers and have the potential as drug carriers and imaging agents.

1. INTRODUCTION

Over the past few decades, there has been considerable interest in developing biodegradable efficiency, rapid leakage of water-soluble drug in the nanoparticles (NPs) as effective drug delivery devices. Various polymers have been used in drug stability. On the other hand, polymeric NPs offer delivery research as they can effectively deliver the some specific advantages over liposomes. NPs help to increase the stability of drugs /benefit, while minimizing side effects (Nilesh Patankar and Dawn waterhouse, 2012). The proteins possess useful CR properties. Controlled release (CR) of pharmacologically active Nanoparticles generally vary in size from 10 to 1000 nm. The drug is dissolved, entrapped, encapsulated or attached to a NP matrix and depending upon major goal in designing such devices.

Irinotecan is a semisynthetic, antitumor drug extracted from plants such as *Camptotheca acuminata*, a derivative of camptothecin alkaloid. Irinotecan acts as a prodrug, which is converted to a biologically active metabolite SN-38 by a carboxylesterase-converting enzyme. Irinotecan serves as a water-soluble precursor of the lipophilic metabolite SN-38. It is thousand-fold more potent than its parent compound irinotecan (Jarko Rautio et al., 2008 and Ron H.J.Mathijsen et al., 2001). SN-38 is highly bound to plasma proteins (approximately 95% bound). Irinotecan and its active metabolite, SN-38, inhibit the action of topoisomerase I, an enzyme that produces reversible single-strand breaks in DNA during DNA replication. These single-strand breaks relieve torsional strain and allow DNA replication to proceed. Irinotecan and SN-38 bind to the topoisomerase I-DNA complex and prevent relegation of the DNA strand, resulting in double-strand DNA breakage and cell death. Irinotecan is cell cycle phase-specific (S-phase) (Ron H.J.Mathijsen et al., 2001).

Nanoparticles are a collective name for nanospheres and nanocapsules. Nanospheres have a matrix type structure, where active compounds can be adsorbed at their surface, entrapped or dissolved in the matrix. Nanocapsules have a polymeric shell and an inner core. In this case, the active substances are usually dissolved in the core, but may also be adsorbed at their surface. Nanoparticles or colloidal carriers have been extensively investigated in biomedical and biotechnological areas, especially in drug delivery systems for drug targeting because their particle size (ranging from 10 to 1000 nm) is acceptable for intravenous injection.

In recent years, microspheres, liposomes, and biodegradable polymers have been used in site-specific drug delivery systems. Hydrophilic-hydrophobic diblock copolymers exhibit amphiphilic behavior and form micelles with core-shell architecture. The hydrophobic block forms the inner core, which acts as a drug incorporation site, especially for the hydrophobic drugs. The hydrophilic block forms the hydrated outer shell, which plays a role in preventing uptake by the reticulo-endothelial system (RES). Nanoparticles made from poly(γ-benzyl L-glutamate) (PBLG) and poly(ethylene oxide) (PEG) are hydrophilic-hydrophobic diblock copolymers which have these predominant characteristics . Thus, this PEG-PBLG co-polymeric carrier may serve as an appropriate vehicle for drug delivery.

The anticancer activity of camptothecin (CPT) and its natural and synthetic analogs has been shown in a broad spectrum of cancers, including leukemias and cancers of the liver, stomach, breast, and colon. Among natural CPTs, 10-hydroxycamptothecin (HCPT) has been shown to be more active and less toxic; however, natural HCPT is in a lactone form and is water-insoluble. One way to improve the solubility of HCPT is to change the lactone form to the

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PRODRUG:

A prodrug is a pharmacological substance that is administered in an inactive (or less than fully active) forms, and is subsequently converted to an active drug through normal metabolic processes (bioactivation). A prodrug serves as a type of 'precursor' to the intended drug. Prodrugs can be used to improve the pharmacokinetic property; they are mainly designed to improve bioavailability.

CAMPTOTHECIN

Camptothecin (CPT) is a cytotoxic quinoline alkaloid which inhibits the DNA enzyme topoisomerase I. It was isolated from the bark and stem of Chinese tree Camptotheca acuminate, CPT showed remarkable anticancer activity in preliminary clinical trials and it also possess low solubility and (high) adverse drug reaction.

SOLVENT EVAPORATION

Solvent evaporation is one of the microencapsulation method widely employed in pharmaceutical industry for the better encapsulation of hydrophobic drugs, to enhance the solubility and dissolution.

DSC:
DSC is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. It is used to observe more subtle physical changes, such as glass transitions. It is widely used in industrial settings as a quality control instrument due to its applicability in evaluating sample purity and for studying polymer curing.

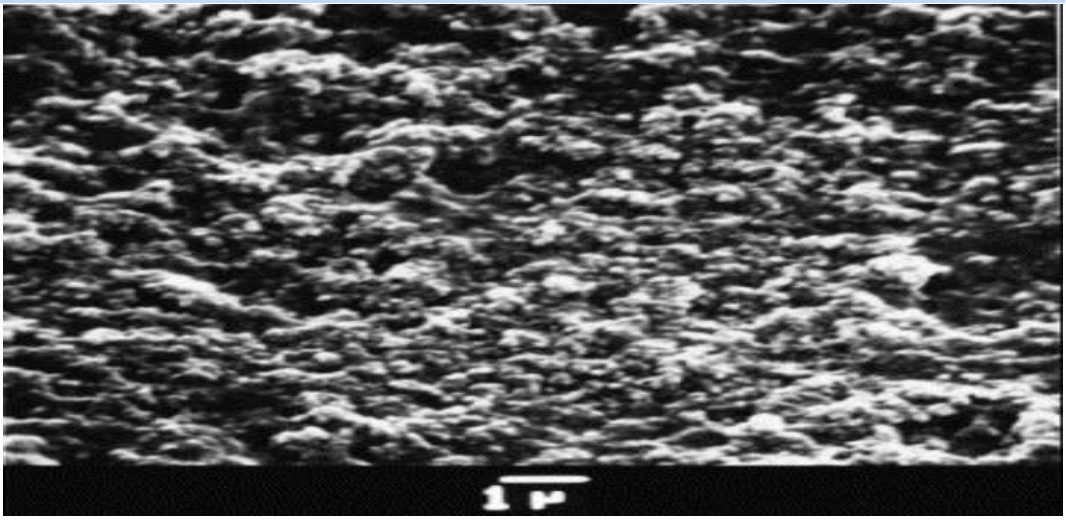

Figure 1
SEM photograph of Raw material

SEM:
A scanning electron microscope (SEM) is a type of electron microscope that images a sample by scanning it with a beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition etc.

Table 1 Composition of Irinotecan HCl nanoparticle Formulations

INGREDIENTS	FORMULATION		
	F1	F2	F3
Irinotecan Hydrochloride	20 mg	20 mg	20 mg
PEG – 400	20 mg	40 mg	60 mg
Pluronic F-68 (Poloxamer)	1.5 mg	1.5 mg	1.5 mg
Dichloromethane	2.4 ml	2.4 ml	2.4 ml
DM water	117.6 ml	117.6 ml	117.6 ml

carboxylate form by adding NaOH. However, this leads to less activity and more unwanted toxicity. At the same time, HCPT has a short half-life in vivo and poor bio distribution. To improve the solubility of CPT analogs, the lactone form of the analogs was incorporated into liposomes or nanoparticles. These delivery systems show favorable pharmacokinetics and bio distribution. In the present study, we prepared HCPT-loaded PEG-PBLG nanoparticles and investigated the *in vitro* release, pharmacokinetics, and anticancer effect. Our results showed that HCPT-loaded nanoparticles changed the pharmacokinetic behavior of HCPT *in vivo*. The HCPT-loaded nanoparticles had a more sustained release, a longer circulation time, increased delivery to tissue, and an enhanced anticancer effect.

2. MATERIALS AND METHODS

2.1. Materials

Irinotecan Hydrochloride was purchased from Shilpa medicare Ltd, (Raichur, India), PEG – 400 was obtained from Sigma aldrich pvt. Ltd, (Bangalore, India), Pluronic F68 (Poloxamer) was obtained from Signet Corporation, (Mumbai, India); Dichloro methane was purchased from Thermo Fisher scientific India pvt. Ltd, (Mumbai, India).

2.2. Methods

2.2.1. Formulation of nanoparticles

Irinotecan loaded nanospheres were prepared by the emulsification solvent evaporation method (Sethi et al., 2010; Priyanka Pandya et al., 2008), The hydrophobic drug Irinotecan (20 mg) and biodegradable polymer poly ethylene glycol were dissolved in an organic solvent methylene chloride of 2.4 ml. This resultant solution was dispersed in an aqueous phase ie. DM water (117.6 ml) containing pluronic F68 (2mg) by using probe sonicator for 2

Table 2 In-vitro drug release profile of PEG nanoparticles of Irinotecan Hydrochloride Formulation (F1 – 1:1)

S.No	Time (h)	Absorbance	Concentration (mg)	Cumulative % of Drug Release
1.	0	0	0	0
2.	1 hr	0.0921	1.87	9.35
3.	2 hrs	0.1208	2.45	12.25
4.	4 hrs	0.1836	3.72	18.6
5.	6 hrs	0.1849	3.75	18.75
6.	8 hrs	0.1926	3.90	19.5
7.	10 hrs	0.2103	4.26	21.3
8.	12 hrs	0.2289	4.64	23.2
9.	14 hrs	0.2684	4.82	23.92
10.	16 hrs	0.2458	4.98	24.9
11.	20 hrs	0.2566	5.20	26.0
12.	24 hrs	0.3842	7.78	38.9

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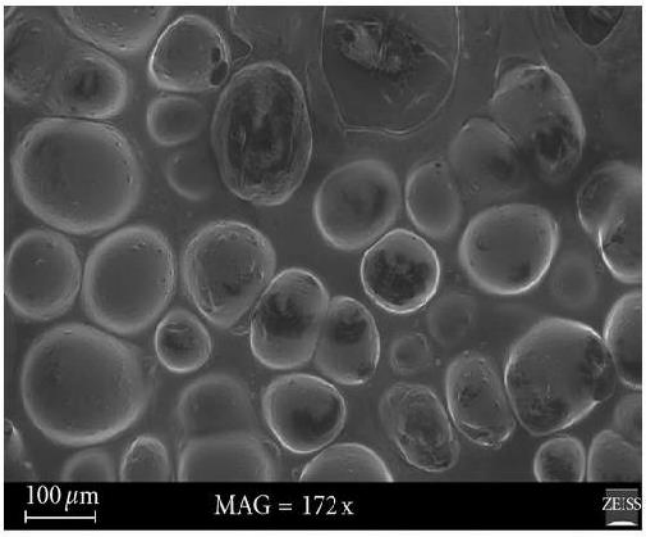

Figure 2
SEM Photograph of Formulation F3

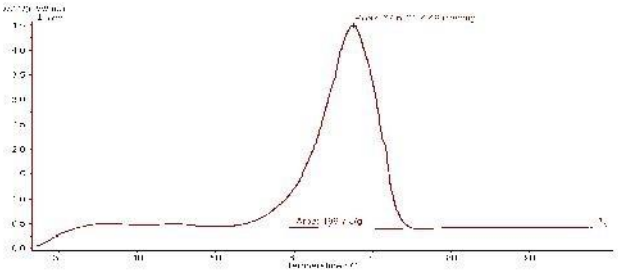

Figure 3
DSC peak for the drug Irinotecan

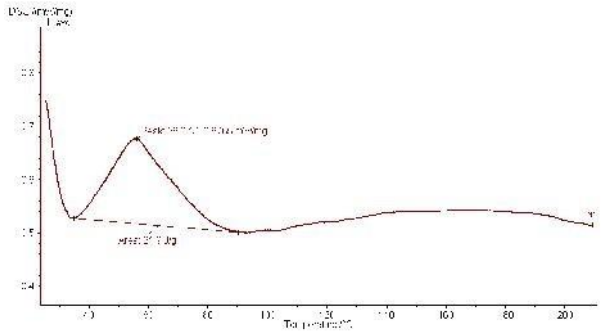

Figure 4
DSC peak for the drug Irinotecan + Polymer PEG

mins, there after the organic solvent was evaporated at 600 rpm for 2 hrs at room temperature using magnetic stirrer, now the formulation can be considered as F1. Then the aqueous suspensions were concentrated in a low pressure system to final volume of 50 ml and filtered in a 0.8 µm Millipore membrane following the above mentioned procedure the formulation of F2 and F3 was carried out by keeping 20 mg of Irinotecan HCl as constant and changing the concentration of polymer as 40 mg and 60 mg (Table 1).

3. RESULTS AND DISCUSSION

3.1. Scanning Electron Microscopy (SEM)

Surface morphology of Irinotecan Hydrochloride nanoparticle formulations were studied by Scanning Electron Microscopy (SEM) operating at 5 keV. A thin layer of the formulations were dispersed on polycarbonate 0.05 µm filter membrane placed on the carbon adhesive tape in an aluminium stub. The samples were dried and then coated with platinum using auto fine coater. Then the scanning electron microphotographs were taken by selecting the field, which consists of spot size setting of 1.0 mm and a working distance of 5 mm[4] (Fig.1 and Fig.2).

3.2. Differential Scanning Colorimetry (DSC)

DSC analysis is widely used to study the interaction between drugs and polymers in the solid state. DSC thermogram of physical mixture is only the superposition of irinotecan (CPT-11) with PEG. No characteristic Irinotecan (CPT-11) endothermic curve is observed in DSC thermograms for CPT-11.PEG complex (Fig.3 and Fig.4).

3.3. In-vitro release studies

The in- vitro studies of the three batches of Irinotecan Hydrochloride formulations (F1, F2, and F3) were carried out using dialysis membrane in a beaker containing 100 ml of phosphate buffer saline (PBS) pH 7.4 maintained at constant room temperature and sink conditions, stirring was carried out at 50 rpm using a magnetic stirrer. The samples were collected at regular intervals of 15, 30, 45, 1, 2, 4, 6, 8, 14, 24 hrs and analyzed by using UV spectrophotometer for

Table 3 In-vitro **drug release profile of PEG nanoparticles of Irinotecan Hydrochloride Formulation (F2 – 1:2)**

S.No	Time	Absorbance	Concentration (mg)	Cumulative % of Drug Release
1.	0	0	0	0
2.	1 hr	0.3125	6.33	31.65
3.	2 hrs	0.3256	6.60	33.0
4.	4 hrs	0.3396	6.88	34.4
5.	6 hrs	0.3896	7.89	39.45
6.	8 hrs	0.4256	8.62	43.1
7.	10 hrs	0.4352	8.82	44.1
8.	12 hrs	0.4852	9.83	49.15
9.	14 hrs	0.5112	10.20	52.26
10.	16 hrs	0.5285	10.71	53.55
11.	20 hrs	0.5812	11.78	58.9
12.	24 hrs	0.5996	12.15	60.75

Table 4 In-vitro drug release profile of PEG nanoparticles of Irinotecan Hydrochloride Formulation (F3 – 1:3)

S.No	Time	Absorbance	Concentration (mg)	Cumulative % of Drug Release
1.	0	0	0	0
2.	1 hr	0.1563	3.17	15.35
3.	2 hrs	0.2986	6.05	30.25
4.	4 hrs	0.4659	9.44	47.2
5.	6 hrs	0.4986	10.10	50.5
6.	8 hrs	0.5216	10.57	52.85
7.	10 hrs	0.6252	12.67	63.35
8.	12 hrs	0.7325	14.84	74.2
9.	14 hrs	0.7401	14.99	74.95
10.	16 hrs	0.7758	15.72	78.6
11.	20 hrs	0.7802	15.94	79.72
12.	24 hrs	0.8025	16.26	81.3

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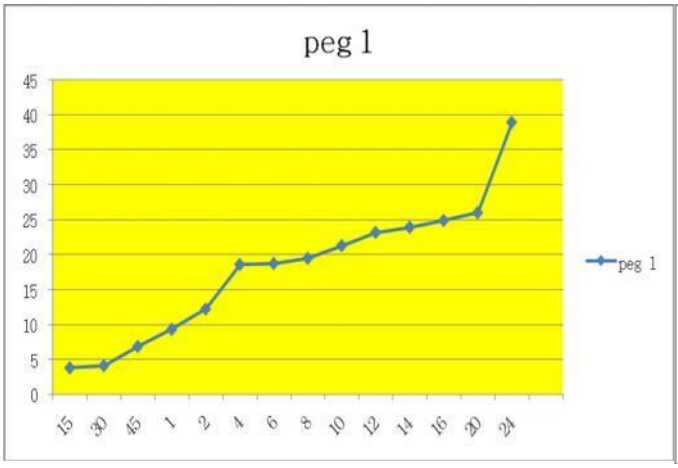

Figure 5

In-vitro drug Release profile of PEG nanoparticle of Irinotecan Hydrochloride Formulation (F1 – 1:1)

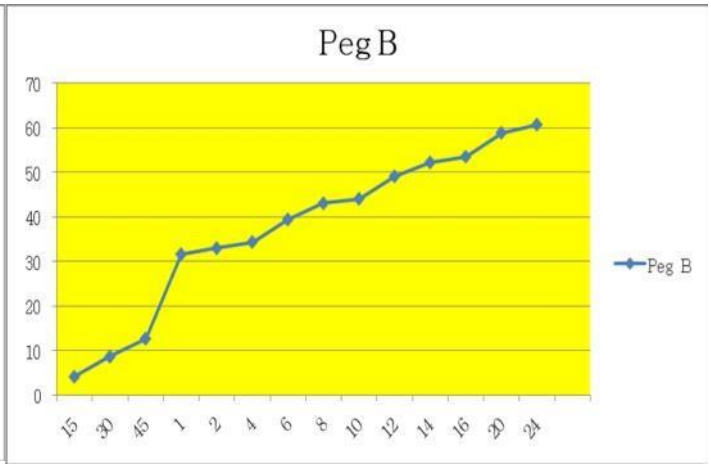

Figure 6

In-vitro drug Release profile of PEG nanoparticle of Irinotecan Hydrochloride Formulation (F2 – 1:2)

absorbance at 254nm[4] and the results are tabulated in Table 2, 3 and 4.

Emergence of Nanotechnology in the recent years in the field of chemotherapy has provided many advantages to the investigators and researchers to overcome certain problems such as the poor aqueous solubility of the drug, improvement in bioavailability, drug targeting, etc. The SEM photograph reveals (Figs.5,6,7 and 8) the anatomy along with their size of the formed nanoparticles. Therefore it is suitable for nanoparticulate drug delivery system, which achieves site specific action. Because of the smaller size it gets rapidly absorbed, which leads to increased bioavailability and in turn provides better therapeutic effect. The in-vitro release study reveals, it is a bi-phasic pattern of drug release, in which 50% of the total loaded drugs from the formulation get released within 5 hrs interval of time and the remaining amount of the drug is again extended for about 19 hrs in a 24 hrs release study. The In-vitro drug release is in the manner of prolonged action, this is because of its nanoparticle nature of the carrier molecule (PEG) combining with the drug which is having a greater effect on drug delivery.

4. CONCLUSION

From the carried work, it has proved that poly ethylene glycol can be combined with the drug Irinotecan HCl for the formulation to have better performance. It is clearly demonstrated that the solvent evaporation method is suitable for poly ethylene glycol (PEG) nanoparticle preparation. Decreasing the concentration of surfactant Pluronic F- 68 leads to increase in size of the particle thereby decrease in polydispersity. This study has shown that poly ethylene glycol are not only well known carrier molecule, and also a powerful tool in drug targeting because they can increase dramatically the loading capacity of nanoparticles. For these reasons Irinotecan HCl with poly ethylene glycol nanoparticles are likely to constitute a promising system for improving intravenous delivery of Irinotecan HCl in the treatment in colorectal cancer. The formulation shows better drug release performance upto 24 hrs for about 81 %. The comparative in-vitro drug release profile of all the three batches showed the controlled drug release, the formulation F3 shows better percentage of drug release in 24 hrs release study. It is clearly concluded that formulation F3 is the best among the three batches while comparing with F1 and F2.

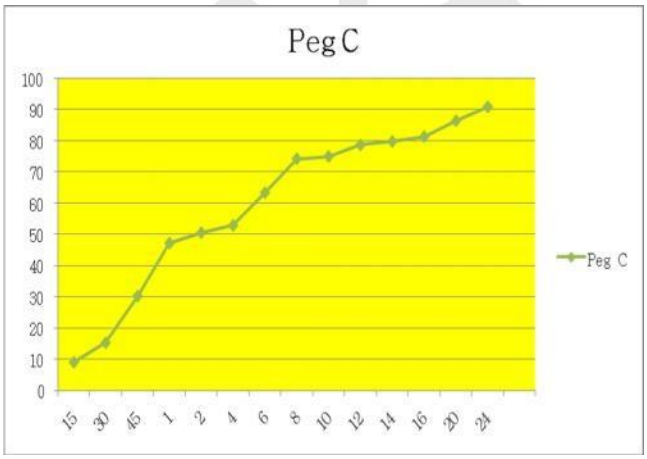

Figure 7

In-vitro drug Release profile of PEG nanoparticle of Irinotecan Hydrochloride Formulation (F3 – 1:3)

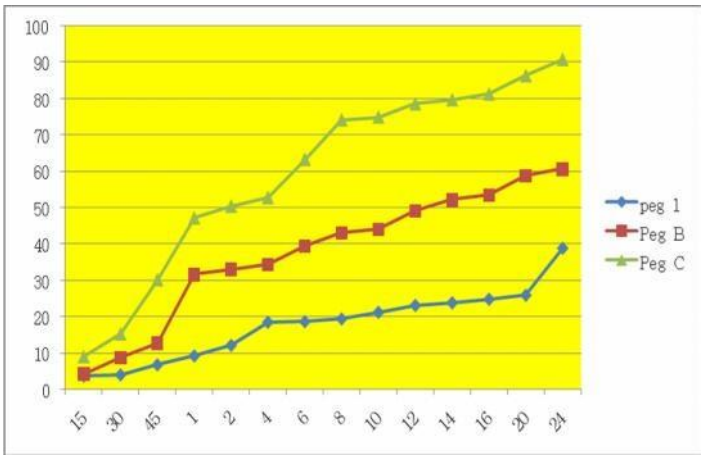

Figure 8

Comparative Release kinetics profile of PEG nanoparticle of Irinotecan Hydrochloride Formulation (F1, F2, and F3 – 1:1, 1:2, and 1:3)

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SUMMARY OF RESEARCH

The polymer PEG can be used in formulation of drug Irinotecan to have a better release, on the targeted site. Use of PEG nanoparticles can increase the encapsulation efficiency of a drug, and they have a prolonged circulation time. Emulsification and solvent evaporation is a s uitable method for the preparation of PEG nanoparticles. SEM photographs reveal the smaller size of formed nanoparticles along with their anatomy. In-Vitro release study showed that the release of drug is in controlled manner and the prepared nanoparticle formulation showed a biphasic pattern of drug release.

FUTURE ISSUES

Recent years have witnessed unprecedented growth of research and applications in the area of nanoscience and nanotechnology. There is increasing optimism that nanotechnology, as applied to medicine, will bring significant advances in the diagnosis and treatment of disease. Anticipated applications in medicine include drug delivery, both in vitro and in vivo diagnostics, nutraceuticals and production of improved biocompatible materials. NPs have a relatively large (functional) surface which is able to bind, adsorb and carry other compounds such as drugs, probes and proteins. However, many challenges must be overcome if the application of nanotechnology is to realize the anticipated improved understanding of the patho-physiological basis of disease, bring more sophisticated diagnostic opportunities, and yield improved therapies. Although solid NPs may be used for drug targeting, when reaching the intended diseased site in the body the drug carried needs to be released. So, for drug delivery biodegradable nanoparticle formulations are needed as it is the intention to transport and release the drug in order to be effective in treating various types of cancer and brain disorders. Potential nano drugs will work by very specific and well-understood mechanisms, one of the major impacts of nanotechnology and nanoscience will be in leading development of completely new drugs with more useful behavior and less side effects.

DISCLOSURE STATEMENT

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